IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re SCHMALJOHN AND HOOPER

Appln. No.: 09/491,974   Group Art Unit: 1632

Filed: January 27, 2000   Examiner: J. Woitach

Title: DNA Vaccine Against Hantavirus Infection

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RULE 132 DECLARATION

Hon. Commissioner of Patents
          And Trademarks
Washington, D.C. 20231

Sir:

I, Jay Hooper, a citizen of the United States of America, am an inventor of the invention identified in the above-referenced application for patent, and I declare and say that:

1. I conducted experiments, or supervised experiments done at my direction, that demonstrated that DNA vaccines expressing the full-length M gene products G1 or G2 <u>alone</u> fail to elicit neutralizing antibody responses and fail to protect against hantavirus infection.

2. To test the hypothesis that a DNA vaccine expressing either G1 alone or G2 alone could confer protective immunity, hamsters were vaccinated 3-4 times with DNA vaccine plasmids expressing only the Hantaan virus G2 protein or only the Hantaan G1. Sera from vaccinated hamsters were tested for neutralizing antibodies using a plaque reduction neutralization test. Vaccinated hamsters were challenged with Hantaan virus. Four weeks after challenge sera was collected and assayed for the presence of anti-nucleocapsid antibodies, which serve as surrogate markers for evidence of Hantann virus infection. The results are summarized in Table 1. As shown there, none of the hamsters

vaccinated with plasmids expressing a single glycoprotein elicited neutralizing antibodies and the levels of protection were no greater than negative controls.

Table 1 . Plasmids expressing a single Hantaan glycoprotein do not elicit NAb and do not protect.

DNA vaccine plasmid	Schedule[1]	NAb positive/ total hamsters	Challenge virus[2]	# Protected/total[3]	# Neg. control[4] protected/total
pWRG/HTN-M(G2 only)	0,3,3	0/5	HTNV	0/5	0/4
pWRG/HTN-G1	0,3,3,8	0/5	HTNV	1/5	1/7
pWRG/HTN-M(G2 only)	0,3,3,8	0/6	HTNV	1/6	1/7

[1] weeks between gene gun vaccinations

[2] Hantaan virus (HTNV) 2000 pfu injected intramuscular

[3] Protection from infection as measured by absence of anti-nucleocapsid response.

[4] Negative control hamsters were vaccinated with empty vector.

pWRG/HTN-M(G2 only) = plasmid containing full M gene but only expressing G2.

pWRG/HTN-G1 = plasmid containg Hantaan G1 sequence, expresses Hantaan G1.

NAb = neutralizing antibody as measured by plaque reduction neutralization test.

3. I also conducted experiments, or supervised experiments done at my direction, that demonstrated that DNA vaccines expressing the full-length M gene products G1 and G2 <u>together</u> do provide homotypic and heterotypic protection of mammals challenged with a hantavirus.

4. As shown in Tables 2 and 3, the PMED vaccine provides, with certain exceptions, homotypic and heterotypic protection of hamsters to challenge with virus. A compilation of the protection data is shown in Table 3.

Table 2
Hantavirus M g ne-based DNA vaccines in hamsters: pr tecti n
against h motypic and heterotypic hantaviruses (by experiment)

DNA vaccine plasmid[a]	Schedule[b]	Challenge virus[c]	# Protected/ total[d]	# Neg. control protected/ total[e]	p[f]
pWRG/SEO-M	0,4,4	SEOV	5/5	0/5	
	0,2,2	SEOV	3/6	0/6	
	0,3,3	SEOV	6/6	0/9	
	0,3,3	SEOV	8/8	0/8	
			22/28 (79%)		1.8E-10
	0,3,3	HTNV	¾	0/5	
	0,3,3	HTNV	7/9	0/9	
			10/13 (77%)		3.4E-05
	0,3,3	DOBV	7/10 (70%)	1/9	0.0149
	0,3,3	PUUV	1/5 (20%)	1/5	0.7778
pWRG/HTN-M	0,3,3	HTNV	8/8 (100%)	0/8	0.0001
pWRG/HTN-M(x)	0,3,3	HTNV	8/8 (100%)	0/8	0.0001
	0,3,3	SEOV	5/7 (71%)	0/7	0.0105
	0,3,3	DOBV	5/7 (71%)	0/8	0.0070
	0,3,3	PUUV	1/8	1/8	
	0,3,3	PUUV	0/8	0/8	
			1/16 (6%)		0.7581
	0,3,3	ANDV	7/16 Lethal	1/8	
	0,3,3	ANDV	2/8 Lethal	0/7	
			9/24 (38%)		0.0569
pWRG/AND-M	0,3,3	ANDV	1/8 Lethal	2/8	
	0,3,3	ANDV	1/8 Lethal	1/8	
			2/16 (13%)		0.8337

[a] pWRG/SEO-M = plasmid expressing Seoul virus G1 and G2; pWRG/HTN-M = plasmid expressing Hantaan virus G1 and G2; pWRG/HTN-M(x) = identical to pWRG/HTN-M except 100 nucleotides removed from vector; pWRG/AND-M = plasmid expressing Andes virus G1 and G2.

[b] weeks between vaccinations

[c] Challenged with ~1000 ID_{50} of SEOV, HTNV, DOBV, and PUUV, or 250 LD_{50} of ANDV.

[d] For challenge with SEOV, HTNV, DOBV, and PUUV protection against infection; for ANDV, protection against a lethal challenge.

[e] Negative controls were vaccinated with an empty vector, or vector containing irrelevant gene, or nothing.

[f] p = one-tailed Fisher's exact test comparing status (protected/non-protected) by treatment group (treated/controls).

SEOV = Seoul virus; HTNV = Hantaan virus; DOBV = Dobrava virus; PUUV = Puumala virus; ANDV = Andes virus.

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Table 3 Hantavirus M gene-based DNA vaccines in hamsters:: protection against homotypic and het rotypic hantaviruses (summary)

Vaccine	% of vaccinated hamsters protected[a]				
	SEOV	HTNV	DOBV	PUUV	ANDV
pWRG/SEO-M	79*	77*	70*	20	nd
pWRG/HTN-M or HTN-M(x)	71*	100*	71*	6	35**
pWRG/AND-M[b]	nd	nd	nd	nd	0
(-) control	0	0	6	10	19

[a] For SEOV, HTNV, DOBV, and PUUV protection is measured by absence of evidence of infection 28 days after challenge; for ANDV, protection from a lethal infection.
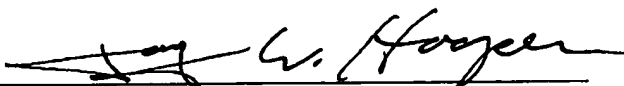[b] pWRG/AND-M was not immunogenic in hamsters.
* Significant level of protection relative to negative control group.
** Suggestive level of protection relative to negative control group.
(-) negative
nd = not done

5. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

_____ Jay Hooper

_6/26/03_ Date